Suppression of Verticillium Wilt of Eggplant by Earthworms

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ABSTRACT

The role of earthworms in plant disease has received little attention. To address whether earthworms would affect the severity of Verticillium wilt of eggplant (Solanum melongena) in the field, we grew eggplants in experimental field plots that were naturally infested with Verticillium dahliae in 2005, 2006, and 2007. Three earthworm treatments were compared: (i) no treatment (untreated control), (ii) earthworm populations reduced via chemical eradicants (carbaryl or hot mustard) (reduced treatment), and (iii) earthworm populations increased by addition of adult Canadian nightcrawlers (Lumbricus terrestris, 11 earthworms per m²) (augmented treatment). Compared to the untreated control, the estimates of the area under the disease progress curve (AUDPC) were reduced while estimates of the canopy growth curve (CGC) and the final plant weights were increased in plots augmented with earthworms in all 3 years. In 2 out of 3 years, eggplant yield (weight and number of fruit) was increased in plots augmented with earthworms. When a carbaryl drench was used to reduce earthworm numbers, the treatment resulted in plants with more disease than in the untreated controls. In 2005 and 2006, carbaryl-treated plants had larger CGC values and higher yield than in the untreated controls and were not significantly different from the augmented plots. When a hot mustard extraction procedure was used to reduce earthworm densities in 2007, plant growth, yield, and disease variables did not differ from the untreated control. Although the effects of reducing earthworms were variable and difficult to explain, our findings suggest that augmenting earthworm populations can suppress Verticillium wilt of eggplant, and strategies that increase earthworm numbers may contribute to disease suppression.

Verticillium wilt, caused by Verticillium dahliae Kleb., can be a major constraint on eggplant (Solanum melongena L.) production in the northeastern United States. Symptoms include wilt, chlorosis, defoliation, small leaves, and stunted plants. There are no highly resistant cultivars (16). Management options, such as fumigation and crop rotation, provide partial disease suppression, but they are not always desirable or practical. Previous research found that using black plastic mulch and ammonium sulfate fertilization provided more suppression of Verticillium wilt of eggplant than bare ground or application of calcium nitrate (14). Although the fertilization practices are available to conventional growers, they are not commonly used by organic growers. Organic growers typically use cover cropping and green manuring to reduce damage from Verticillium wilt. Regardless, any growers with Verticillium-infested fields who want to transition to organic practices can suffer major losses in the first few years until cover cropping and green manuring reduce damage from soilborne diseases (34,36,42).

Earthworm activity is an important component of soil health (1,25). Charles Darwin (5) was one of the first to show their role in plant decomposition. Subsequent research showed that earthworm burrowing and processing of organic matter improves nutrient availability (35) and facilitates percolation of water into lower soil horizons (13,19,44). Over the past decade, some studies have shown that earthworms were associated with disease suppression (4,6,27,33). Vermicomposts, which are end-products of the breakdown of organic matter by earthworms, are desirable or practical. Previous research found that earthworm populations in soil infested with fungal pathogens would suppress disease. The objectives of our report were to determine if augmenting Verticillium-infested soils with adult earthworms would affect the yield, plant growth, and development of Verticillium wilt of eggplant.

MATERIALS AND METHODS
Plot establishment. Eggplant cv. Black Beauty (Comstock Ferre Seed Co., Wethersfield, CT) seeds were germinated in the greenhouse in soilless potting mix in 36-cell trays and fertilized twice with 100 ml of 20-10-20 (N-P-K) Peter’s soluble fertilizer (1.0 g/liter) (Scotts Inc., Lincoln, NE). One-month-old seedlings were used in all field experiments. Adults of L. terrestris were purchased from a fishing supply house (N.A.S. Inc., Marblehead, OH) each year. Earthworms were kept at 10°C for no more than 4 weeks. Earthworms were rinsed three times in tap water before being used.

Field trials were conducted during the 2005, 2006, and 2007 growing seasons at Lockwood Farm in Hamden, CT, on a Cheshire fine sandy loam (Typic Dystricrept) (pH 6.1). The field had been planted horizontally.
to eggplants and other solanaceous hosts for over 20 years and had high populations of *V. dahliae*. Prior to the initiation of this study, 15 soil cores were removed to a depth of 15 cm, bulked, and three samples were removed and assayed for *V. dahliae* by the method of Huisman and Ashworth (18), which estimated 13 ± 5 microscelidrotia per g soil. The fields were cover-cropped each year with winter rye and plowed under on 1 June 2005, 2 June 2006, and 28 May 2007. Different parts of the field were used each year to avoid any residual effects from the treatment of the previous year(s).

In 2005 and 2006, square plots (2.1 × 2.1 m) were trenched and lined with clear, rigid, 20 gauge, polyvinylchloride (PVC) plastic film (1.27 mm thickness, 70 cm wide) (EPV Plastics Inc., Oxford, MA) to contain the earthworms. Trenches were dug approximately 36 cm deep so that the plastic vertically extended 30 cm out of the plot. Since horizontal movement of *L. terrestris* occurs mostly on the soil surface, the PVC plastic was sufficient to contain the earthworms in each plot. Plots were planted on 8 June 2005 and 10 June 2006 with four eggplant transplants that were evenly spaced 1.2 m apart, one in each quadrant, approximately 0.6 m from the plot edge. Three treatments were applied within 3 days of planting: (i) augmented earthworm treatment, (ii) reduced earthworm treatment, or (iii) untreated control. There were four replicate plots per treatment. Earthworm densities were augmented by supplementing plots with 75 adult earthworms (11 earthworms per m²). The soil surface was irrigated to break up soil surface crusts. Earthworms were individually added to the soil surface approx- mately 15 cm apart in a uniform pattern where they immediately began burrowing. For the reduced earthworm treatments, plots were drenched with car- baryl (1.1 mg a.i./plot) applied as 50 ml of Sevin SL (Bayer Crop Science, Research Triangle Park, NC) in 20 liters of water. Since *L. terrestris* feeds on plant residues on the soil surface, drenching the soil surface with the toxic carbaryl prevented earthworms from emerging from their burrows and feeding, and reduced their presence and activity in these plots. No attempt was made to determine how deep the chemical treatment penetrated the soil. This treatment was presumed effective in reducing earthworm activity since no cast- ings and middens were observed in these plots during the season, whereas they were readily apparent in the other treatments. In 2005, all plots except the reduced treatments were covered the next day with 133 liters of composted leaf mold to suppress weeds and provide a food base for the earthworms. Weeds were removed weekly by hand. In 2006, all plots received com- posted leaf mold the day after treatments were applied. In both years, all plots re- ceived 22.6 kg of dehydrated cow manure (Agway Inc., North Haven, CT), applied 3 to 4 weeks after planting.

In 2007, the experimental treatments and design were altered. Plots were not separated by PVC film. Since the surface migration of *L. terrestris* is usually less than 1 to 2 m, provided sufficient surface residues were present to discourage long-distance feeding forays (22), we spaced plots 1.2 m apart with untreated borders between the treated plots. Plots (3.75 × 1.2 m) consisted of four plants spaced 0.75 m apart in rows spaced 1.2 m apart. There were five replicate plots per treatment arranged in a randomized block design. Plants were planted on 1 June 2007. Poly- propylene landscape fabric (DeWitt Sunbelt, Silkeston, MO) was laid down be- tween all plots allowing 5 cm between the plants and the landscape fabric. In addition, the reduced treatment was altered. Earthworms were removed from plots with a hot mustard soil extraction method (21).

Although the removal of all earthworms is an unrealistic and unobtainable goal, this procedure was comparable to extracting earthworms by digging and sieving the soil (21). It is nonlethal to earthworms and is environmentally nontoxic. One hundred twenty grams of hot mustard powder (McCormick & Company, Inc., Sparks, MD) was agitated in 1 liter of deionized water for 2 h to dissolve the active compo- nent, allyl isothiocyanate. The slurry was then added to 12 liters of tap water to yield a 10% suspension (wt/vol) that was poured onto the soil surface (3.0 m long × 0.6 m wide) from a watering can. The landscape fabric was temporarily removed during the extraction. Approximately 15 min later, the treated plots were covered with fabric to allow more of the suspension to pene- trate the soil. As earthworms emerged from their burrows, they were removed, rinsed in water, counted, and distributed elsewhere. A mean of 15.6 (78 total) and 17.0 (82 total) earthworms were removed from each treated plot on 7 June and 20 July 2007, respectively. Seventy-five adult earthworms were applied to plots design- nated as augmented (11 earthworms per m²) as described before. Weeds that grew in between the landscape fabric were re- moved by hand.

**Plant growth and disease measure- ments.** Beginning 3 weeks after planting, plant canopies were measured for disease severity and canopy size every 1 or 2 weeks for 9 weeks in 2005, 9 weeks in 2006, and 6 weeks in 2007 as described previously (14). Disease severity (%) was visually assessed by estimating the per- centage of the attached foliage exhibiting symptoms of chlorosis and wilt. Since symptomatic leaves of eggplant rapidly defoliate and can leave holes in the canopy where sunlight passes through, we esti- mated canopy size and the fraction of ground cover observable from overhead to provide another estimate of plant health. Briefly, the elliptical shape of an eggplant canopy (A, m²) was estimated using the formula A = [(L × W)/4] × π, where L = length (m) and W = width (m) of the cano-opy measured from directly overhead with a meter rule. The fraction of groundcover was determined by visually estimating the fraction of the soil surface that could be seen within the elliptical shape of the cano-opy (S). Canopy size (m²) was then calcu- lated in the equation: canopy size = A × (1 − S). The area under the plant canopy growth curve (CGC, m² days) (or area under the disease progress curve [AUDPC, disease days]) was computed (14). When symptoms first appeared on leaves of a plant, pieces of wilting petioles were surface-disinfested in 0.053% sodium hy- pochlorite for 1 min, rinsed, and placed onto ethanol water agar supplemented with streptomycin sulfate (250 mg/liter) (26). Colonies of *V. dahliae* that grew from the petioles confirmed the presence of the pathogen, and no further isolations were made from plants in that plot. Marketable fruit were harvested, counted, and weighed every 4 to 5 days from late July through mid-September. At the final harvest, all remaining fruit were removed and weighed. The plant was cut at the soil line and weighed. There was no attempt to remove and measure root weights.

**Statistical analyses.** Each year, the ex- periments were arranged in randomized complete block designs. The experimental treatments were the same in 2005 and 2006, so the data from both years were analyzed for interactions between years and presented separately for each year if significant interactions were detected. Treatment effects were tested using SYS- TAT V.10 (Cranes Software International Limited, Bangalore, Karnataka, INDIA) procedure for mixed model ANOVA with year and replication as random effects. The 2007 data were analyzed for treatment effects with replication as random effects. Means were separated using Tukey’s test at *P* < 0.05.

**RESULTS**

There were significant interactions be- tween treatment and year for CGC, AUDPC, and the yield components, so data for each year are presented separately. The treatment × year interaction was a result of significantly more disease in un- treated plots in 2006 than in 2005 (*P* < 0.01; Table 1). In 2005, plants grown in the augmented earthworm treatment had ap- proximately twice the value for CGC, fruit numbers, fruit weight, and final plant weights than the untreated controls (Table 1). The AUDPC values were significantly less in the augmented treatment than in untreated controls (*P* < 0.001). The growth and yield variables were greater in the carbaryl drench compared to the untreated control (*P* < 0.001) and did not differ from
the augmented treatment. However, plants in the reduced treatment had significantly larger AUDPC values than the untreated controls. The effect of the augmented earthworm treatment on disease severity in 2005 was an overall reduction in symptom expression during the season when compared to the untreated and reduced treatments (Fig. 1).

The augmented earthworm treatments resulted in similar trends in 2006 even though there was more disease in the untreated controls (Table 1). Compared to the untreated control, the augmented treatment increased the CGC, fruit number, fruit weight, and final plant weights and also reduced the AUDPC value. Eggplants in the reduced treatment had lower CGC values and final plant weights than the untreated control, but did not differ in yield or AUDPC values. The main effect of the augmented treatment on disease in 2006 was a delay in the onset of symptom expression when compared to the untreated plots (Fig. 1).

In 2007, when the experimental design was changed, eggplant plots augmented with earthworms still had larger CGC values and larger final plant weights than controls ($P < 0.05$), but there were no significant effects on yield (Table 1). The augmented treatment reduced the AUDPC values relative to the untreated controls. Plots that received the reduced treatment using the hot mustard extraction method did not differ in disease severity, growth, or yield from the untreated controls. As in 2006, the reduced treatment significantly delayed the onset of symptoms (Fig. 1).

**DISCUSSION**

The current study found that manually augmenting *Verticillium*-infested field plots with adult earthworms resulted in increased eggplant growth and yield in 2 out of 3 years, and reduced disease estimates of *Verticillium* wilt in all 3 years. Although the mechanism(s) governing disease suppression that could be attributed to earthworms was not addressed in this study, a previous greenhouse study found that earthworms increased population densities of fluorescent pseudomonads and filamentous actinomycetes (15). These microbes previously have been implicated in disease suppression (23,27,37). Earthworm burrows are filled with microbially rich castings (29,32,40,41). Presumably, rapidly growing roots would occupy these burrows and become exposed to high densities of beneficial organisms in earthworm castings. These microbes may suppress disease by one or more of many different mechanisms, such as competition, antagonism, and/or induced resistance (23,38). Earthworm activity also increases the availability of nutrients that affect plant health, which in turn affects disease suppression (7,24,31,35). Since one study found that the viability of microcroroseteria of *V. dahliae* was not reduced after passage through the gut of the earthworm (28), it is unlikely that earthworms reduced inoculum. Given these reports, disease suppression is likely mediated through a microbial mechanism.

In both 2005 and 2006, yields were higher in augmented plots compared to untreated plots, but this was not observed in 2007. The earthworm-augmented treatment increased the CGC values and the final plant weights in 2007, but there was no corresponding increase in yield. One explanation could be that the earthworm activity in 2007 increased canopy size, but did not produce an increase in flowers or perhaps the flowers developed normally and then aborted. The authors have noticed that eggplants on occasion would abort their flowers without any observable cause, but still retain a healthy canopy (W. H. Elmer and F. J. Ferrandino, *unpublished data*). Tedeschi and Zerbi (39) found that high soil water potential was associated with eggplants that aborted their flowers. Although we did not measure soil water potential, this explanation cannot be supported by any large difference in the weather since cumulative rainfall for 2007 was 37.7 cm, whereas it was 26.8 and 58.3 cm for 2005 and 2006, respectively. In addition, the minimum/maximum temperature means for each month of the growing season were similar among years.

Applying carbaryl to plots resulted in unexpected improvements in plant growth and yield. Since total earthworm numbers are difficult to monitor, it is not known if densities were reduced enough to have an effect, but the lack of middens and surface castings in this treatment suggested there was minimal activity. Roberts and Dorough (30) classified carbaryl as extremely toxic to earthworms (LC$_{50} = 1 – 10 \mu g/cm^2$), and Cathey (3) considered it to be one of the most lethal insecticides available. We know of no reports that demonstrate that carbaryl can suppress *Verticillium*, and since the AUDPC values in the current study indicated significant disease was still present, we conclude that carbaryl did not suppress *Verticillium* wilt. In 2007, when earthworm populations were reduced by hot mustard extraction, we did not see any reduction in yield compared to the untreated control. Although more than 160 earthworms were manually extracted from these plots, it is still not clear if numbers were reduced enough to impact growth and disease. It is possible that another yield-limiting pest was being suppressed by the carbaryl applications, but we have identified no candidates. We know of no reports that suggest carbaryl could affect soil fauna in a manner that could directly or

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**Table 1.** Effect of augmenting or reducing earthworm (*Lumbricus terrestris*) densities on eggplant growth, yield, and area under the disease progress curve of *Verticillium* wilt in the field in 2005, 2006, and 2007

<table>
<thead>
<tr>
<th>Year</th>
<th>Treatment*</th>
<th>Canopy growth curve (m$^2$ days)*</th>
<th>Number of fruits</th>
<th>Eggplant yield (kg/plant)*</th>
<th>Final plant weights (g)*</th>
<th>AUDPC* (disease days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>Untreated</td>
<td>10.1 a*</td>
<td>5.7 a</td>
<td>2.08 a</td>
<td>533 a</td>
<td>398 b</td>
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<td></td>
<td>Reduced</td>
<td>17.7 b</td>
<td>11.2 b</td>
<td>4.56 b</td>
<td>945 b</td>
<td>623 a</td>
</tr>
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<td></td>
<td>Augmented</td>
<td>20.6 b</td>
<td>12.0 b</td>
<td>4.70 b</td>
<td>1,071 b</td>
<td>242 c</td>
</tr>
<tr>
<td>2006</td>
<td>Untreated</td>
<td>8.3 a</td>
<td>3.4 a</td>
<td>1.69 a</td>
<td>465 a</td>
<td>612 a</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>10.0 b</td>
<td>4.5 ab</td>
<td>1.78 a</td>
<td>572 b</td>
<td>689 a</td>
</tr>
<tr>
<td></td>
<td>Augmented</td>
<td>10.5 b</td>
<td>5.0 b</td>
<td>2.30 b</td>
<td>581 b</td>
<td>474 b</td>
</tr>
<tr>
<td>2007</td>
<td>Untreated</td>
<td>3.5 a</td>
<td>8.2 a</td>
<td>2.02 a</td>
<td>966 a</td>
<td>320 a</td>
</tr>
<tr>
<td></td>
<td>Reduced</td>
<td>3.7 a</td>
<td>9.4 a</td>
<td>2.27 a</td>
<td>944 a</td>
<td>264 ab</td>
</tr>
<tr>
<td></td>
<td>Augmented</td>
<td>4.8 b</td>
<td>7.4 a</td>
<td>2.09 a</td>
<td>1,055 b</td>
<td>221 b</td>
</tr>
</tbody>
</table>

* Plots were drenched with carbaryl in the reduced treatment in 2005 and 2006, and earthworms were manually extracted with a 10% hot mustard suspension in 2007. Adult earthworms (75 per plot) were added in the augmented treatment.

* Canopy growth curve (m$^2$ days); an integrated estimate of the plant canopy size (dm$^2$) measured weekly.

* Plots were 7 m$^2$ and contained four plants.

* Fresh weight of the aboveground growth.

* Area under the disease progress curve based on weekly estimates of disease severity.

* Values represent the mean of four replicate plots (16 plants) for 2005 and 2006 and five replicate plots (20 plants) for 2007; values followed by differing letters are significantly different according to Tukey’s test at $P = 0.05$. 

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indirectly affect Verticillium wilt of eggplant. Flea beetles (*Epitrix fuscula* Crotch) caused damage early in the season, but we have discounted any effect of carbaryl on these pests since it was applied as a soil drench and carbaryl is not systemic.

The current study poses the question: Is earthworm augmentation too expensive for growers to use? Since *L. terrestris* is very difficult to rear (2), most distributors obtain their earthworms from collectors who manually harvest the species from open lawns and golf courses (43). At the current cost of 5 to 7 cents per earthworm and an application rate of 5 to 10 earthworms per m², augmenting a field would cost an unacceptable $2,500 to $7,000 per ha. Extracting and collecting earthworms from nearby pastures might serve as an alternative, but even this approach might be unacceptable on large acreage. Growers transitioning to organic farming should consider other management strategies that favor earthworms including liming, winter cover cropping, green manuring, minimum tillage, and minimizing chemical inputs. However, new technological advances in encapsulating earthworm cocoons for large-scale delivery into furrows of agronomic crops (Advanced Prairie Inc., Elliott, IL, U.S. Patent 5127186) may allow large and small growers to quickly increase earthworm densities provided the application is affordable. Our findings suggest that strategies to increase earthworm numbers and activity may, in turn, contribute to disease suppression.

**LITERATURE CITED**


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**Fig. 1.** Effect of earthworms (*Lumbricus terrestris*) on the development of Verticillium wilt symptoms (% disease severity) of eggplants in plots infested with *Verticillium dahliae*. Treatments were: Augmented (adult earthworms added [11 m²]), Reduced (treated with applications of carbaryl [2005 and 2006] or hot mustard suspensions [2007] to reduce earthworm densities), or Untreated. Error bars represent standard error of the mean (*P* = 0.05).


